REMARKS

The following remarks are in response to the Examiner's Office Action mailed on June 25, 2004 and Applicants' telephone interview with Examiner Lori A. Clow, Ph.D., on October 7, 2004. Claims 23-26 have been canceled Claims 1 and 12 have been amended. Claims 1-22 are pending.

Applicants express their appreciation to the Examiner for conducting the telephone interview. During the interview, Applicants discussed the claimed invention with the Examiner and distinguish the claims from the teaching of U.S. Patent No. 6,232,104 (Lishanski et al.) cited by the Examiner in the rejection of the claims under 35 U.S.C. §103(a).

The claimed invention relates to a method of detecting a difference in two related nucleic acid sequences, such as single nucleotide polymorphism (SNP), through the formation of a four-way complex (e.g., a Holliday Junction (HJ)). Compared to the method taught in Lishanski et al., the claimed invention has much improved sensitivity of the detection, especially for the detection of SNPs in short nucleotides.

Applicants discovered that introduction of extra mismatches into one or more nucleic acids can improve the sensitivity of genotyping methods. Page 15, lines 10-13. As specified in independent claims 1 and 12 as amended, extra mutations have been introduced to the reference (or target) polynucloetide by design. For example, PCR forward primers were designed in which a T was substituted for a naturally occurring A at different positions other than the targeted SNP position (Page 34, lines 10-13), so that the resulting amplicons containing the mismatches, when present in the 4-way complex, would create an energy barrier to migration of the four-way complex. Such an energy barrier presumably would increase the likelihood that a difference at a site of a polymorphism will impede migration of the complex, resulting in a more stable four-way complex and increased levels of the complex to facilitate detection. Page 15, lines 23-27. As evidenced in the Example section of the Specification, introduction of additional mismatches greatly increased HJ formation in short amplicons. *See*, e.g., Example 4.

In contrast, Lishanski et al. teaches detecting a difference between two related nucleic acid sequences (e.g., single nucleotide polymorphism) using a pair of partial duplexes that are **identical except for the targeted difference.** See *Abstract,* and Figure 1A and its legend.

According to Lishanski et al., in Figure 1A partial duplexes A' and B' are related in that their hybridized portions are identical except for mutation M in partial duplex A'. Column 12, lines 49-51. The mutation M is the targeted site with a difference between the two related partial duplexes A' and B'. Thus, Lishanski et al. does not teach the claimed method that uses a pair of partial duplexes with the target or reference sequence that is **designed to have a mutation not at the targeted site of the polymorphism.**

Instead, Lishanski et al. suggests that in order to detect a difference between the target and reference sequences, the sequences other than the targeted site need to remain identical. Lishanski et al. cautioned that creating two partial duplexes that are entirely different would destroy the purpose of the invention—detection of mutation in the target nucleic acid. Specifically, Lishanski et al. teaches that

> Since the target-related double-stranded portions of the quadramolecular complex produced from the combination of partial duplexes produced by specific and non-specific priming are **entirely different**, such complexes cannot exchange strands and dissociate into labeled full duplexes. The stable quadramolecular complexes are detectable, and thus generate a signal that is related to **non-specific priming but not to the presence of a mutation.**

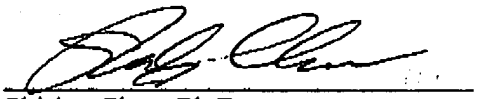
Column 26, lines 57-64, emphasis added. Thus, Lishanski et al. fails to motivate one of ordinary skill in the art to design a target or reference sequence that has a mutation not at the targeted site of polymorphism in order to detect SNP at the target site.

None of the secondary references, Wu et al., Kumar et al., and Giesen et al., supplies the claim elements missing in Lishanski et al. As acknowledged by the Examiner, Wu et al. merely teaches introduction of a GC-rich sequence; Kumar et al. a duplex with minor groove binding motif; and Giesen et al. duplexes with PNAs. In view of the failure of the cited references to teach or suggest all of the claim elements, Applicants submit that a prima facie case of obviousness has not been established under 35 USC 103(a). Withdrawal of the rejection is therefore respectfully requested.

CONCLUSION

In light of the remarks and arguments set forth above, Applicants earnestly believe that they are entitled to a letters patent, and respectfully solicit the Examiner to expedite prosecution of this patent application to issuance. Should the Examiner have any questions, the Examiner is encouraged to telephone the undersigned.

Respectfully submitted,

Date: October 12, 2004 By: 

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